

# Dynabeads® Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T Cell Kit

Catalog no. 11363D

Store at 2°C to 8°C

Rev. Date: February 2012 (Rev. 005)

## Kit Contents

Kit contents	Volume
Depletion MyOne™ Dynabeads® (Regulatory T Cell Kit)	2 × 5 mL
Antibody Mix Human CD4 (Regulatory T Cell Kit)	2 × 1 mL
Dynabeads® CD25 (Regulatory T Cell Kit)	1 × 5 mL
DETACHaBEAD® (Regulatory T Cell Kit)	1 × 2 mL

### Kit capacity

MNC: ~1 × 10<sup>9</sup>

For details on product content, see “Description of Materials” section.

## Product Description

This product is intended for magnetic isolation of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells from human mononuclear cells (MNCs). The CD4<sup>+</sup>CD25<sup>-</sup> cell fraction can be used as effector cells in downstream inhibitory assays. This kit isolates highly pure CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells that express the intracellular transcription factor FOXP3. The isolated cells are highly pure, viable, and bead-free. In the first step, the non-CD4<sup>+</sup> MNC are labeled with Antibody Mix Human CD4. In the second step, Depletion MyOne™ Dynabeads® are added to remove the non-CD4<sup>+</sup> MNCs. In the third step, Dynabeads® CD25 are added to the CD4<sup>+</sup> T cells to capture the CD4<sup>+</sup>CD25<sup>+</sup> T cells. In the fourth and last step, Dynabeads® CD25 are removed from the cells.

**Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

## Downstream Applications

Isolated cells may be used directly in any downstream application including flow cytometry and cell expansion protocols (e.g. using Dynabeads® Treg expander for regulatory T cell stimulation and expansion).

For research use only. Not for human or animal therapeutic or diagnostic use.

- It is important to remove all DETACHaBEAD® reagent from the isolated cells (step 26 in the protocol) to avoid wrong scatter in the flow cytometer.

## Protocol

Approximately 3–10% of the CD4<sup>+</sup> T cell population expresses the CD25 antigen. This protocol describes magnetic labeling and isolation of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells from 1 × 10<sup>8</sup> MNCs using Dynabeads® Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T Cell Kit. When working with fewer cells than 1 × 10<sup>8</sup>, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

## Wash the Beads

See Table 1 and 2 for volume recommendations.

1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of beads to a tube.
3. Add the same volume of Isolation Buffer from step 2, or at least 1 mL, and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed beads in the same volume of Isolation Buffer as the initial volume of beads (step 2).

## Prepare Cells

Prepare a MNC suspension according to “General Guidelines”. Resuspend the cells at 2 × 10<sup>8</sup> cells/mL in Isolation Buffer.

## Isolate Untouched CD4<sup>+</sup> T cells

1. Transfer 500 µL (1 × 10<sup>8</sup>) PBMC in Isolation Buffer to a tube.
2. Add 200 µL heat inactivated FBS/FCS and 200 µL Antibody Mix Human CD4.
3. Mix well and incubate for 20 min at 2°C to 8°C.
4. Wash the cells by adding 4 mL Isolation Buffer. Mix well by tilting the tube several times and centrifuge at 350 × g for 8 min. Discard the supernatant.
5. Resuspend the cells in 2 mL Isolation Buffer.
6. Add 1 mL pre-washed and resuspended Depletion MyOne™ Dynabeads®.
7. Incubate for 15 min at 18°C to 25°C with gentle tilting and rotation.
8. Add 3 mL Isolation Buffer.
9. Resuspend the bead-bound cells thoroughly by pipetting >10 times using a pipette with a narrow tip opening. Avoid foaming.
10. Place the tube in the magnet for 2 min. Transfer the supernatant containing the untouched human CD4<sup>+</sup> T cells, to a new tube.
11. *Optional:* To remove residual beads; place the tube in the magnet for 2 min and transfer cells to a new tube.
12. Spin down the cells at 350 × g for 8 min and resuspend the cells in Isolation Buffer to 1.5 × 10<sup>7</sup> CD4<sup>+</sup> T cells/mL.

## Isolate CD4<sup>+</sup>CD25<sup>+</sup> Cells

This protocol is based on 1.5 × 10<sup>7</sup> CD4<sup>+</sup> T cells/mL, but is directly scalable according to Table 2.

13. Add 200 µL pre-washed and resuspended Dynabeads® CD25 per 1.5 × 10<sup>7</sup> CD4<sup>+</sup> cells.
14. Mix well and incubate for 25 min at 2°C to 8°C with rolling and tilting.
15. Place the tube in the magnet for a minimum of 1 min. Carefully remove the supernatant containing the CD4<sup>+</sup>CD25<sup>-</sup> (effector) cells.
16. Remove the tube from the magnet and carefully resuspend the bead-bound cells in 5 mL Isolation Buffer by gently shaking the tube instead of pipetting the cells.

## Required Materials

- Magnet (DynaMag™ portfolio). See [www.lifetechnologies.com/magnets](http://www.lifetechnologies.com/magnets) for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Isolation Buffer: Ca<sup>2+</sup> and Mg<sup>2+</sup> free phosphate buffered saline (PBS) supplemented with 0.1% BSA and 2 mM EDTA. **Note:** BSA can be replaced by human serum albumin (HSA) or 2% FBS/FCS.
- RPMI with 1% FBS.
- *Optional:* Flow cytometry antibodies. We recommend using anti-CD3 clone UCHT-1 from Caltag Medsystems as primary fluorescent antibody for flow staining of cells after isolation. *Optional* clones: OKT3, H1TA3.
- *Optional:* For viability analysis, SYTOX® Red is recommended.

## General Guidelines

- Visit [www.lifetechnologies.com/cellisolation](http://www.lifetechnologies.com/cellisolation) and follow our QuickLinks for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that beads do not settle in the tube.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.
- To avoid unspecific labeling of cells during flow staining, we recommend using gammaglobulin prior to staining with primary fluorescent antibody.

17. Place the tube in the magnet for a minimum of 1 min. Remove and discard the supernatant.
18. Wash one more time by carefully resuspending the cells in 5 mL Isolation Buffer and gently shaking the tube.
19. Place the tube in the magnet for a minimum of 1 min. Remove and discard the supernatant.
20. Resuspend the bead-bound cells in 500 µL RPMI with 1% FBS.

### Release of CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T cells

21. Add 80 µL DETACHaBEAD<sup>®</sup> reagent and incubate for 45 min at room temperature with tilting and rotation.
22. Place the tube in the magnet for a minimum of 1 min. Carefully remove the supernatant containing the CD4<sup>+</sup>CD25<sup>+</sup> cells to a new tube.
23. Wash the Dynabeads<sup>®</sup> CD25 twice in 1 mL RPMI with 1% FBS to obtain the residual cells and collect the supernatant after separation on a magnet.
24. Add 5 mL RPMI with 1% FBS to wash the cells, followed by centrifugation at 350 × g for 8 min. Remove all visible liquid without disturbing the pellet. Repeat step 23.
25. Discard the supernatant and resuspend the cells in a preferred cell culture medium.

The isolated CD4<sup>+</sup>CD25<sup>+</sup> cells are pure, viable and bead-free, and ready to be used in any downstream assay.

Table 1: Recommended volumes for "Isolate Untouched human CD4<sup>+</sup> T cells"

Step	Step description	Volumes per 1 × 10 <sup>8</sup> PBMC	Volumes per 5 × 10 <sup>8</sup> PBMC
	Recommended tube	5–7 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Cell volume	500 µL	2.5 mL
2	FBS/FCS	200 µL	1 mL
2	Antibody Mix	200 µL	1 mL
4*	Wash cells (Isolation Buffer)	~4 mL	~20 mL
5	Resuspend cells (Isolation Buffer)	2 mL	10 mL
6**	Depletion MyOne™ Dynabeads <sup>®</sup>	1 mL	5 mL
8*	Increase volume	~3 mL	~15 mL

Table 2: Recommended volumes for "Isolate CD4<sup>+</sup>CD25<sup>+</sup> cells" and "Release of CD4<sup>+</sup>CD25<sup>+</sup> cells"

Step	Step description	Volumes per 1 × 10 <sup>8</sup> PBMC	Volumes per 5 × 10 <sup>8</sup> PBMC
	Recommended tube	5–7 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-5	DynaMag™-50
13	Volume cells	1 mL	5 mL
13**	Dynabeads <sup>®</sup> CD25	200 µL	1 mL
16–18*	Wash Dynabeads <sup>®</sup> CD25 (Isolation Buffer)	2 × ~5 mL	2 × ~10 mL
20	Resuspend cells (RPMI with FBS)	500 µL	2.5 mL
21	DETAHaBEAD <sup>®</sup> reagent	80 µL	400 µL
23	Wash Dynabeads <sup>®</sup> CD25 (RPMI with FBS)	2 × 1 mL	2 × 5 mL
24*	Wash cells (RPMI with FBS)	~2 × 5 mL	~2 × 15 mL

\* Adjust the Isolation Buffer volumes to fit to the tube you are using. \*\* Transfer the sample to a smaller tube during the release to avoid cell loss on the tube wall.

\*\* When incubating, tilt and rotate the vial so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

## Description of Materials

Depletion MyOne™ Dynabeads<sup>®</sup> contains ~20 mg beads/mL uniform, superparamagnetic polystyrene beads (1 µm diameter) coated with a monoclonal, Fc-specific, anti-human IgG. Dynabeads<sup>®</sup> CD25 contains ~4 × 10<sup>8</sup> beads/mL (~30 mg/mL) uniform, super-paramagnetic polystyrene beads (4.5 µm diameter) coated with monoclonal mouse anti-human CD25. All beads are suspended in PBS pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. Antibody Mix Human CD4 contains the monoclonal mouse anti-human IgG antibodies CD8, CD14, CD16 (specific for CD16a and CD16b), CD19, CD56, CD36, CDw123, and CD235a (Glycophorin A) supplied in PBS and 0.02% sodium azide.

## Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer <sup>®</sup> Sample Mixer	15920D
SYTOX <sup>®</sup> Red	S34859

**REF** on labels is the symbol for catalog number.

## Limited Use Label License

The purchase of this product conveys to the purchaser the limited, nontransferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com) or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

## Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.lifetechnologies.com/termsandconditions](http://www.lifetechnologies.com/termsandconditions). If you have any questions, please contact Life Technologies at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

SPEC-06051

©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners, except where otherwise stated. LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

For support visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) or email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

[www.lifetechnologies.com](http://www.lifetechnologies.com)

